Enhancing the Sensitivity and Stability of Biosensors by Novel Nanostructures

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Introduction and Objectives

Recently there has been an increasing demand and interest to develop implantable glucose sensors for treating diabetes. Despite many notable progresses in this area, there are key issues related to implantable glucose sensors, including biofouling and calibration. Biofouling refers to the rapid accumulation of adsorbed proteins and organisms on the working surface of the sensor, which will in turn reduce the lifespan of the sensor. Calibration concerns are two folds: first, an implanted sensor usually measures the interstitial glucose level, which has to be correlated to the true blood glucose level; second, the biofouling and other aging effects tend to offset the signal measurements of the sensors over time, thus demanding re-calibration time and again after the primary calibration. With this project, we aim to achieve the following specific objectives: (1) to fabricate nanostructures with controlled parameters, such as size, spacing, height, shape and location, for developing nanoelectrodes; (2) to immobilize enzymes onto well-prepared nanostructures to achieve high sensitivity and activity; (3) to control the size and separation of the top layer nanorods to be within 20 nm, so that a mechanical filtration can be realized for antifouling; (4) to passivate the top layer nanorods using self-assembly monolayer (SAM) technique to further improve antifouling; and (5) to fabricate prototype glucose sensors and assess their sensitivity, stability and antifouling behavior.

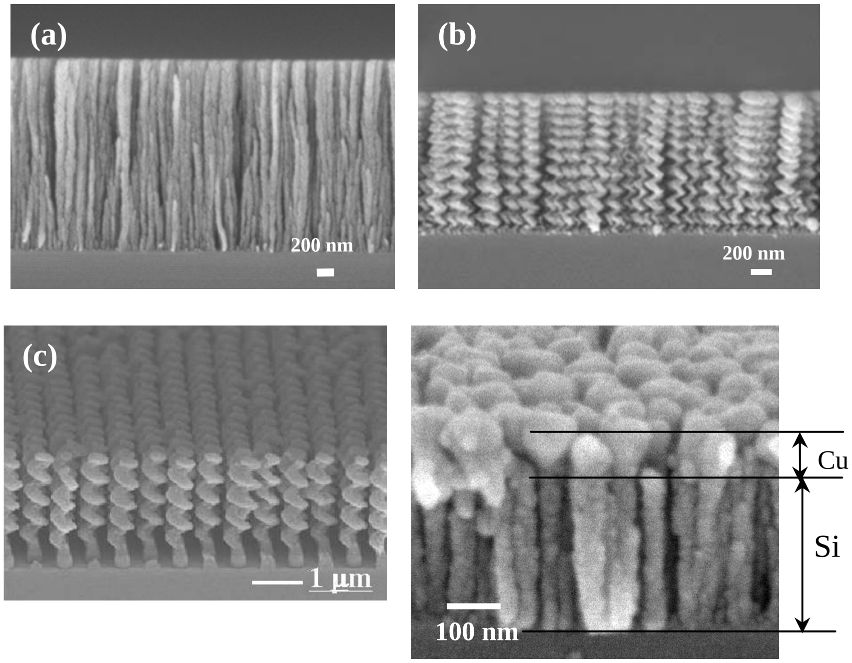

Fig. 1. The cross-section SEM images of three-dimensional nanorods prepared by the GLAD technique: (a) Vertically aligned Si nanorod array; (b) Si nanospring array; (c) Regular array of Si nanospring grown on W-plugs; (d) Cu/Si two-layer nanostructure matrix.

Research Accomplishments

In the past year, our team used a parallel approach and carried out multiple activities in order to simultaneously make progress under each of the above objectives. These activities included nanostructure fabrication, study of liquid-nanostructure interaction, enzyme immobilization on nanostructures, and electrochemical characterization of nanostructures. Activities in each of these areas are summarized below.

Nanostructure fabrication

The main nanofabrication technique applied in our research is called glancing angle deposition (GLAD). GLAD is a physical vapor deposition technique in which the substrate is rotated in the polar and azimuthal directions by two stepper motors programmed by a computer. During deposition, the angle between the incoming vapor from the source and the surface normal of the substrate is set between 0^o to 90^o, and the substrate can rotate azimuthally. The main mechanisms that control the growth are the shadowing effect and surface diffusion. Figure 1 shows some examples of nanostructures that can be fabricated by GLAD. GLAD is simple to implement, and any thin film physical vapor deposition system can be readily changed to a GLAD system. In fact, the most intriguing aspect of GLAD is that the structures of the nanorods can be well-designed by programming the substrate rotations.

In the area of nanostructure fabrication, we successfully produced Si and Ag nanorod arrays using GLAD technique. For Si nanorod arrays, we focused on the following: (a) Control of nanorods separation by using two different incident angles (86^o and 88^o) for GLAD deposition. (b) Fabrication of different structures, namely, vertical-cylindrical nanorods and vertical-helical nanosprings by using a Labview program developed by an undergraduate student. (c) Investigation of the properties of liquid-nanostructure interaction. (d) Implementation of computerized control of the GLAD deposition system. (f) Training all the students in the group on the GLAD fabrication technique. For Ag nanorod arrays, we focused on the following: (a) Study of the growth mechanism of Ag nanorods. (b) Performing electrochemical tests on Ag nanorods electrodes. (c) Measuring the optical properties of the Ag nanorods using surface plasmon resonance and surface enhanced Raman Scattering. In particularly, by slightly modify the GLAD technique, we have successfully deposit aligned nanorod arrays onto optical fibers and other cylindrical objects (Fig. 2). This technique can be used to fabricate nanostructured fiber optical sensors.

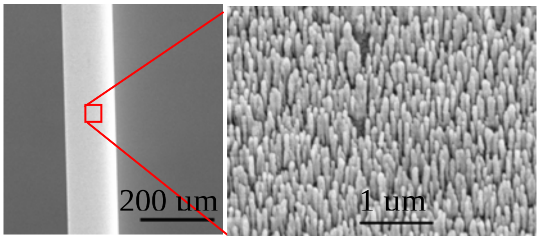

Fig. 2. Aligned Ag nanorods on an optical fiber.

Enzyme immobilization on nanostructures

We performed preliminary studies to compare the activity of functionalized nanostructures via traditional kinetic and end-point enzyme assays. Because of the inability to continuously monitor absorbance changes in 96-well plates in response to peroxidase-catalyzed reactions due to the presence of solid nanostructures in the wells, we performed a correlation study and confirmed that end-point absorbance readings can accurately reflect enzyme activity measured in terms of reaction rates. Following that, we compared end-point absorbance readings of the enzymatic activities for functionalized

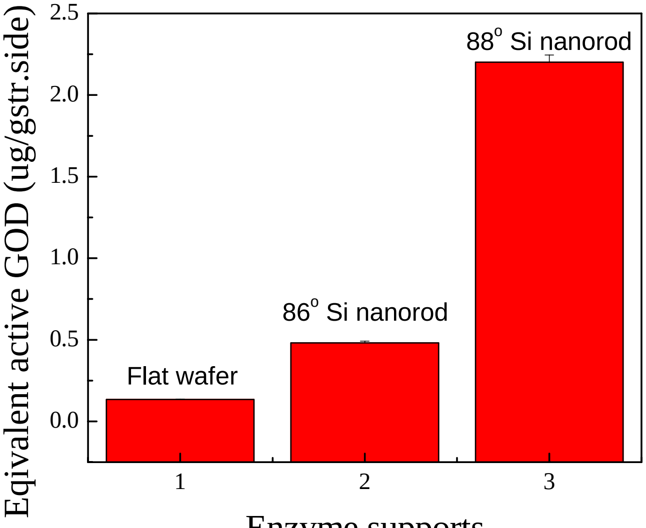

Fig. 3 Activity of GOD immobilized on Si flat and nanorod wafers.

nanostructured and flat substrates. The 10- to 20-fold increase in activity demonstrated in Figure 3 suggests potential to solve the sensitivity problem of functionalized nanorod sensors. Si nanorods prepared by GLAD contain a higher surface are for immobilizing enzymes which in turn results in higher GOD activities. It is important to optimize the system with respect to the nanorod density. At low density, the surface area in not optimal and at high densities, the inter-rod dimensions may be such that the enzyme is too large to penetrate deep into the structure. So far two key challenges her emerged: 1) the loss activity in response to repeat use as depicted in Fig. 4 and 2) the poor mechanical stability of the nanorods. We are addressing these challenges in several ways. For activity stability, we are trying to pinpoint the weakest point in the linker while investigating the possibility of enzyme poisoning.

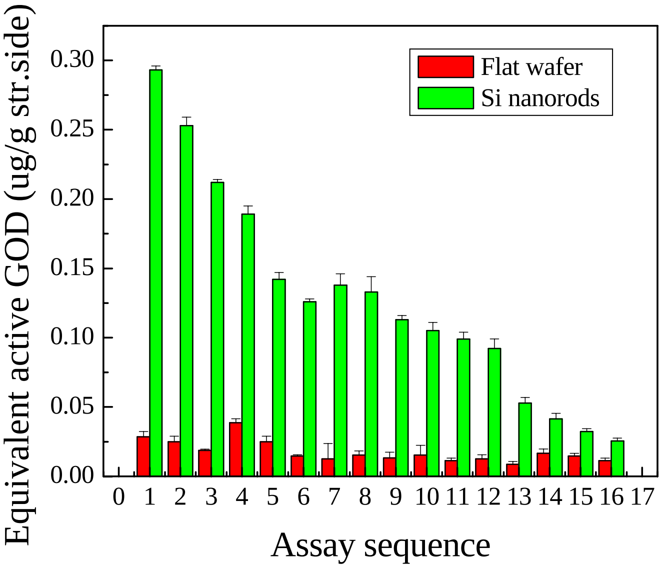

Fig. 4 GOD activity change in response to repeat structure use in end-point assay.

Electrochemical study of nanostructures

We also studied the fundamental electrochemical behavior of the nanostructures. Using the Ag nanostructured substrates as working electrodes in a three-electrode system, we compared the cathodic current responses in nanostructured versus flat substrates. Using Ag nanorod structures as the electrodes and NaCl or PBS as the electrolytes, we studied the electrochemical activities in the cathodic region in order to avoid any unwanted oxidation of Ag. We observed that in NaCl (at 0.1 M and 1M concentrations) Ag nanorod structured substrates exhibited an increased cathodic current response by a factor of 17 as compared to the flat Ag film.

Related publications supported by this project are listed in Ref. [2]-[7]

References

[1] For further information about this project email **zhaoy@physast.uga.edu**, **williamk@engr.uga.edu**, or **gzhang@engr.uga.edu**.

[2] J.-G. Fan, D. Dyer, G.-G. Zhang, and Y.-P. Zhao, "*Nano-Carpet Effect: Pattern Formation during the Wetting of Vertically Aligned Nanorod Array*" Nano Letters **4**, 2133 (2004).

[3] S.-H. Li, X. -F. Zhu, and Y.-P. Zhao, "*Carbon Assisted Growth of* SiO_x *Nanowires*", J. Phys. Chem. B. **108**, 17032 (2004).

[4] Wade Bowie and Y.-P. Zhao, "*Monte Carlo Simulation of Vapor Deposition Polymerization*," Surf. Sci. Lett. **563**, L245 (2004)

[5] Jianguo Fan, Xiaojia Tang, and Y.-P. Zhao, "*Water Contact Angles of Vertically Aligned Si Nanorod Arrays*," Nanotechnology **15**, 501 (2004).

[6] G. Zhang, "*Geometric Nonlinearity in Nanoscale Helical Coils*," Proceedings of the 49th SPIE Annual meeting 2004.

[7] G. Zhang, YP Zhao, W Kisaalita, C Keith, JG Fan, F Haq, D Dyer, G Sawaya, K Uyesugi, "*Nanostructured Terrain for Supporting Neurite Growth*," Transactions of the Society For Biomaterials, Vol. **25**, 727, 2004.